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Characterization of plant growth promotion and disease control trends of rhizobacteria isolated from Umorok (*Capsicum chinense*)

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Abstract
The aim of the study is to identify and characterize the rhizosphere bacteria of King Chili locally known as Umorok plant (*Capsicum chinense*) and evaluates their plant growth promotion and defense from pathogens. A total of 300 rhizobacteria were isolated from three different growth stages of king chili *viz*.; juvenile, flowering and fruiting growth stages and characterized to establish the beneficial role. Nevertheless, the isolated microorganisms were evaluated for their *in-vitro* plant growth promotion potential by performing biochemical tests and pathogen antagonistic activities by dual culture methods. Out of the total isolates, 10 potential rhizobacteria were selected for pot culture experiment based on their multiple Plant Growth Promoting Rhizobacteria (PGPR) and biocontrol activity. Since these inoculants exhibited multiple trait benefits to the host plant, they can be adapted as potential bio-inoculant consortia for plant health improvement and disease control to increase Umorok productivity.

Keywords: PGPR, Umorok, rhizobacteria, bio-inoculant, dual culture, biochemical test

Introduction
Chili is considered as one of the most commercial spice crops, also known as wonder spice. India is the largest producer, consumer and exporter of chili in the world. India shares about 50 to 60 per cent of global production of chili (Geetha and Selvarani 2017) [15]. Manipur's Umorok (*Capsicum chinense*) also known as Naga King Chili, is an indigenous chili variety grown in northeastern India. It has a broad-spectrum ethnopharmacological potential (Meghvansi *et al.* 2010) [26]. Most of the chili species and varieties cultivated in India contain around 1% capsaicin while King chili has around 2–4% capsaicin (Mathur *et al.* 2000) [25]. Very high capsaicin output per unit weight of dry powder renders to lower down the cost of extraction of capsaicin from chili, thereby giving it an upper hand in the national as well as international markets. It shares about 53 percent of agricultural income in hills and 43.9% of farming revenue in plains of Manipur (Malangmeih, Dey and Sagolsem 2015) [24], but it could not provide a stable price and income to the farmers. There are three primary reasons including - lack of improved cultivation methods, crop management, and disease management – explain the limitations in commercialization. Yield potential and sustainability of crop plant typically depends on either tolerance to insect pests or resistance to microbial diseases and that is directly linked to the biological property of soils (Altieri and Nicholls 2003) [4].

Soil is main reservoir for nutrients and potential rhizospheric microflora. The beneficial rhizospheric microbiota so called the Plant growth promoting rhizobacteria (PGPR) have been studied for plant growth and health (Vessey 2003) [38]. PGPR are a diverse group of microorganism living in plant rhizosphere that are increasingly studied for their contributions in increasing agricultural productivity through promotion of growth and triggering of induced systemic resistance (ISR) in plants (Lucy M, Reed E 2004) [21]. PGPR promotes growth and protect from pathogen through various mechanisms like atmospheric nitrogen fixation, phosphate solubilization, phytohormones, siderophore, Hydrogen cyanide (HCN) and hydrolytic enzymes (Babalola OO 2010) [7]. Plant-growth promotion and biocontrol efficacy of PGPR often depend upon the rhizosphere competence of the microbial inoculants in the soil (Lugtenberg and Kamilova 2009) [22]. Rhizosphere competence refers to the survival and colonization potential of PGPR (Bulgarelli *et al.* 2013) [9] and it is thought to be highest for each PGPR strain when associated with its preferred host plant.

This phenomenon was established to some extent why some PGPR strains exhibiting promising bio control agents *in vitro* though they possess variable bio control efficacy in the rhizosphere of a given crop under a given set of conditions.

Earlier studies demonstrated the chili rhizosphere microbial activity importance in various conditions including crop improvement, biocontrolling of pests, and pathogen resistance. The PGPR bacterial isolates showing multiple growth promotion has been using as biocontrol agent for the crop improvement (Datta, Sengupta and Pandit 2010) [12]. *Azotobacter* from chili rhizosphere exhibited an efficient plant growth promotion activity (Kanchana *et al.* 2013) [19], *Aspergillus spp* from chili rhizosphere enhanced the growth of cucumber plant when inoculated with its rhizosphere (Shaikhul *et al.* 2014) [34], and chitinolytic bacillus from chili rhizosphere was used as biocontrol agent for whitefly, *Bemisia tabaci (*Genn.), in chili (Mubarik *et al.* 2010) [28]. Even though chitinolytic bacteri (Suryanto, Patonah and Munir 2010) [36], *Trichoderma viride* (Sastiya *et al.* 2016) were not isolates of chili rhizosphere, but used as biocontrol agent against fusarium wilt, while *Trichoderma spp* controlled the *Pythium ultimum* pathogen (Zagade *et al.* 2012) [40]. Similarly, in umorok, *Trichoderma* and *Pseudomonas* were tested as biocontrol agents for seedling rot causing *Rhizoctonia solani* (Ngullie and Daiho 2013) [29], fruit rot causing *Colletotrichum gloeosporioides* (Ngullie, Daiho and Upadhyay 2010) [30], and bacterial wilt causing *Ralstonia solanacearum* (Jacq *et al.* 2006) [18]; however, the bacteria were not part of the umorok rhizosphere. In this study, the focus was shifted to entire PGPR of umorok, in various aspects that are beneficial to the plant, to extensively explore the important native bacteria that were missed in the previous experiments.

The identification and characterization of PGPR populations indigenous to umorok rhizospheres is therefore critical to discover the strains that can be utilized to improve growth and health in umorok. The objectives of the present study were to isolate bacterial strains from the rhizosphere of umorok to characterize the isolates on the basis of their morphological and physiological attributes as well as by 16S rRNA sequence analysis, and to assess the plant growth promoting effects of the isolates *in vivo.* This study demonstrates the ability of umorok PGPR to promote growth and good health.

Materials & Methods

Soil sample collection

The rhizosphere bacteria sample of umorok was collected from soil in different stages after planting them in the controlled environment. Fresh fruits of Umorok were collected from local farmers of Manipur, and the seeds were sterilized with 70 % ethanol for 5 min and 4% sodium hypochlorite for 10 min. After washing with sterile distilled water, the seed were inoculated on 1% agar medium for sprouting. Sprouted seeds were planted in pots containing non sterile and natural soil. After growing in pot, soil sample and root sample were collected aseptically eliminating the extrinsic microbial contamination of the above ground part (Lundberg *et al.* 2012) [23]. After manually removing the loose soil, the roots were placed in 50 ml tube containing 25 ml sterile distilled water and vortexed to remove the attached soil. Further, roots were sonicated at low frequency for 5 min to disrupt tiny soil aggregates and attached microbes. Then the turbid distilled water was centrifuged at 8000g for 5 min, and the loose pellet was collected. Soil sample thus collected was processed for the isolation of rhizospheric microbiota during juvenile, flowering, and fruiting stages.

Isolation and Molecular characterization of rhizosphere micro-organisms

For isolation of the rhizosphere micro-organisms, 1 g fraction of soil was serially diluted and plated on to the Luria Bertani Agar, King's B Medium, Pikovskaya's Agar, Tryptic Soy Agar, Brain Heart Infusion Agar, and Rojo Congo Medium using standard spread plate method. After 2-3 day incubation at 30 ± 2 °C, morphologically distinct colonies were isolated and sub cultured repeatedly until pure culture colonies were obtained. The cultures were stored in Luria Bertani broth with 30% glycerol at -80 °C and in agar slant at 4°C indefinitely until use. For molecular characterization, the genomic DNA of the cultures was extracted using standard lysozyme method. For sequencing of the 16S rDNA region, genomic DNA was amplified using 63F and 1387R primers.

Biochemical tests

Protease production assay

Skim milk agar (Hi Media, India) was used for protease production estimation. The rhizo bacteria were inoculated on agar plates and incubated at 30˚C ± 2˚C for 4-5 days until the adequate growth appears. If the rhizo bacteria produces a protease enzyme, a clear zone around the colony is being seen. Protease activity was quantified by subtracting the diameter of the bacterial colony from the diameter of the total zone.

Cellulase production assay

Cellulase enzyme production was screened using carboxymethyl cellulose (CMC) following a modified protocol of Aneja (Aneja 2003) [5]. Minimal synthetic media was prepared using 0.2% NaNO3, 0.8% K2HPO4, 0.1%Mg.SO4.7H2O and 0.8% KCl with the addition of 0.2% peptone, 0.1% glucose, and 0.5% CMC. K2HPO4 and CMC solution were prepared separately, by stirring minimal synthetic media continuously, K2HPO4 solution was dissolved first and followed by CMC solution, final pH was adjusted to 5.3. Rhizo bacteria were inoculated on the CMC plates and incubated at 30˚C ± 2˚C for 2-3 days. The CMC plates were flooded with 1% iodine in 0.5% potassium iodide solution for few seconds and drained off to observe a clear halo zone around the colony to determine the positive enzyme production. The zone diameter was determined by subtracting the diameter of the fungal colony from the diameter of the total halo zone.

β- 1, 3-glucanase production assay

β-1, 3-glucanase production was detected using carboxymethyl cellulose agar media that was incorporated with laminarin (Sigma) (Katatny *et al.* 2001) [20]. After incubation at 28˚C ± 2˚C for 4-5 days, the colonies were flooded with a 0.1% congo red dye for 15 min and washed with 1N NaCl and 1N NaOH twice for 15 min respectively. The appearance of a clear zone around the colony indicates positive activity. The zone diameter was determined by subtracting the diameter of the bacterial colony from the diameter of the total clear zone.

Chitinase production assay

Chitin utilization by the rhizobacteria were detected with incorporation of Colloidal chitin and indicator dye

bromocresol purple in the media. The media were prepared with slight modification from the method given by Agrawal (Agrawal and Kotasthane 2009) [2]. After incubation at 30˚C ± 2˚C for 4-5 days, color change from yellow to purple color around the colony indicates positive chitinase activity.

Siderophore Production Assay

The siderophore production was assayed by inoculating the rhizobacteria on Chrome Azurol S (CAS) agar medium and incubating at 30˚C ± 2˚C for 4-5 days. The CAS medium was prepared using a modified protocol of Schwyn and Neilands (Schwyn and Neilands 1987) [33]. For blue agar preparation, 60.5mg CAS was dissolved in 50 ml of distilled water and mixed with 10 ml iron (III) solution (1 mM $FeCl_3.6H_2O$, 10 mM HCl). In 40 ml of distilled water, 72.9mg of hexadecyltrimethylammonium bromide (HDTMA) indicator was dissolved and the CAS-iron solution was added to it with constant stirring. For the basal medium preparation, 0.5% succinic acid, 0.4% K_2HPO_4, 0.4% $(NH_4)_2SO_4.7H_2O$, and 2% agar at pH 5.3 were used and autoclaved at 121◦C for 15 min. Both the blue agar and basal medium were partially cooled, then the blue agar was slowly added to the basal media with proper mixing until it gives the desired blue color agar and finally poured onto the plates. The CAS reaction rate was determined by the color change from blue to yellowish orange, purple or dark purplish red, while non-siderophore producing organisms were distinguished if no color change was observed. The zone diameter was determined by subtracting the diameter of the bacterial colony from the diameter of the total color zone.

Phosphate solubilization assay

Pikovskaya agar with the addition of 0.3% insoluble calcium triphosphate (HiMedia, India) was used for the phosphate solubilization detection (Hilda R. and Fraga R. 2000) [17]. The rhizobacteria were inoculated and incubated at 30˚C ± 2˚C for 4-5 days until growth appears. The presence of halo zone or clear zone around the test PGPR colony, after incubation, ensures indicates phosphate solubilizing potential. Phosphate solubilization was determined by subtracting the diameter of the bacterial colony from the diameter of the total halo zone.

Nitrogen fixation assay

Nitrogen free media was prepared in test tube to assay the growth of the rhizobacteria. The umorok rhizobacteria were stab cultured on the solidified medium in the tube and incubated at 30˚C ± 2˚C for 4-5 days until the desired growth was observed. Under Nitrogen fixing condition, the colour of the media in the tube changes to blue (Hartmann *et al.* 2006) [16]

IAA Production Assay

The IAA production assay was accomplished by following the protocol of Bric *et al*. (Bric, Bostock and Silverstone 1991) [8]. Luria Bertani broth (LB), LB incorporated with 5 mM L-tryptophan (LBT), and LBT incorporated with 0.05% sodium dodecyl sulfate and 1% glycerol was used for screening of potential rhizobacteria for qualitative IAA production. Rhizobacteria were inoculated on the media overlaid with an 82-mm-diameter disk of sterile nitrocellulose membrane and incubated inversely at 30˚C ± 2˚C for 4-5 days until the desired substantial growth was observed. The membrane disc was removed from the plate and treated with Salkowski's reagent. IAA producing cultures form a characteristic red halo zone within the membrane immediately surrounding the colony.

HCN production assay

The production of Hydrogen cyanide was tested using a modified protocol of Miller and Higgins (Miller and Higgins 1970) [27]. A solution of 3% picric acid with 1.5% sodium carbonate was prepared as HCN testing solution. Sterilized strips of Whatman filter paper number 1 were soaked in the HCN testing solution and dried in sterile environment. The rhizobacteria were streaked on tryptic soy agar (TSA) slant, sterile HCN testing solution treated dried paper was placed over the bacteria, to air tight the slant lid was wrapped with parafilm, and the set up was incubated for 4-5 day. The HCN production rate was determined by the color changes in the filter paper strip, from the original yellow to brown or reddish brown color. Scoring was done as weak (yellow to light red), moderate (brown), and strong (reddish brown).

Antagonism Assay

The rhizobacteria were evaluated for their antagonistic activity against widely prevailing soil pathogens *viz*. *Pythium ultimum* (ITCC No. 1650), *Rhizoctonia solani* (ITCC No. 6491), and *Fusarium oxysporum* (ITCC No. 6246). The pathogens were obtained from Indian type culture collection (ITCC), New Delhi, India, for dual culture plate assay (Coskuntuna, A., and Ozer 2008) [11]. About 5mm of pathogen fungal discs were inoculated at the center and the test organism were streaked at four sides with a partition gap of 3cm approximately on the potato dextrose agar (PDA) plates, whereas plates inoculated only with the pathogen served as control. The plates were wrapped with parafilm and incubated at 30˚C ± 2˚C for 2-3 days until the desired growth was observed. Plates inoculated only with the pathogen served as control. The inhibition percentage was calculated using the following formula (Fokkema J, Dickinson and Preece 1976) [14],

$$\text{Inhibition \%} = (C - T)/C \times 100$$

Here, "C" represents the growth diameter of the pathogen in the control plate and "T" represents the pathogen diameter growth on the dual plate, where both the test bacterial and the pathogen were placed.

Greenhouse experiment

The effect of the isolates on umorok was evaluated in pot culture experiment. Inoculum of the cultures was produced in 250 ml LB broth under continuous shaking for 24 h on a rotary shaker at 120 rpm and 30°C. Optical density of the broth was adjusted between 0.3 to 0.5 at 600 nm to obtain a cell concentration of 10^9cfu/ml and applied as a soil drench to the autoclaved soil to reach the inoculum size to a level of 10^7cfu/g soil; For seedling preparation, the seeds were sterilized with 70 % ethanol for 5 min and 4% sodium hypochlorite for 10 min. The seeds were then washed with sterile distilled water until yellowish colour disappears. Washed seeds were soaked in distilled water for 3 – 4 hrs before inoculating on 1 % agar medium at 37 ± 2 °C for sprouting. Sprouted seeds were then planted in pots containing sterile soil inoculated with the selective bio-inoculant to evaluate the effect on growth, however, the plants without any bacterial inoculant were treated as experimental control. Each experiment was performed with three replicates placing the pots in random block design (RBD).

Result

Isolation and characterization of rhizobacteria

A total of around 300 pure cultures were isolated during the three different growth stages of Umorok. The isolated cultures were characterized based on 16s rDNA sequence and grouped into five existing phyla in the systematic microbial classification: actinobacteria, alphaproteobacteria, betaproteobacteria, gammaproteobacteria, and firmicutes. The sequences were submitted to GeneBank database of national center for biotechnology information (NCBI), accession numbers: KY038202-KY038328. Actinobacteria was observed more in juvenile stage (22), however, 32% and 64% reduced in flowering (15) and fruiting (8) stages; similarly, gammaproteobacteria was dominated in juvenile stage (33) as plant transformed into mature stages 15% and 33% of bacterial identity was lost in flowering (28) and fruiting (22) stages; interestingly, no betaproteobacteria was observed during juvenile stage, but, flowering and fruiting stages restored 10 and 16 bacterial varieties. Contrastingly, alphaproteobacteria was increased to 22% more in flowering stage (22) and decreased 17% in fruiting stage (15) when compared to juvenile stage (18); surprisingly, firmicutes were very less in juvenile stage (3) but increased several orders of magnitude in flowering (5) and fruiting (10) stages (Figure 1). Although fluctuation with in the bacterial phyla was observed in each stage, overall number in juvenile (76), flowering (80) and fruiting (71) stages was remain unchanged much. Gammaproteobacteria followed by alphaproteobacteria and actinobacteria were observed more while betaproteobacteria and firmicutes were counted less throughout the umorok plant life cycle.

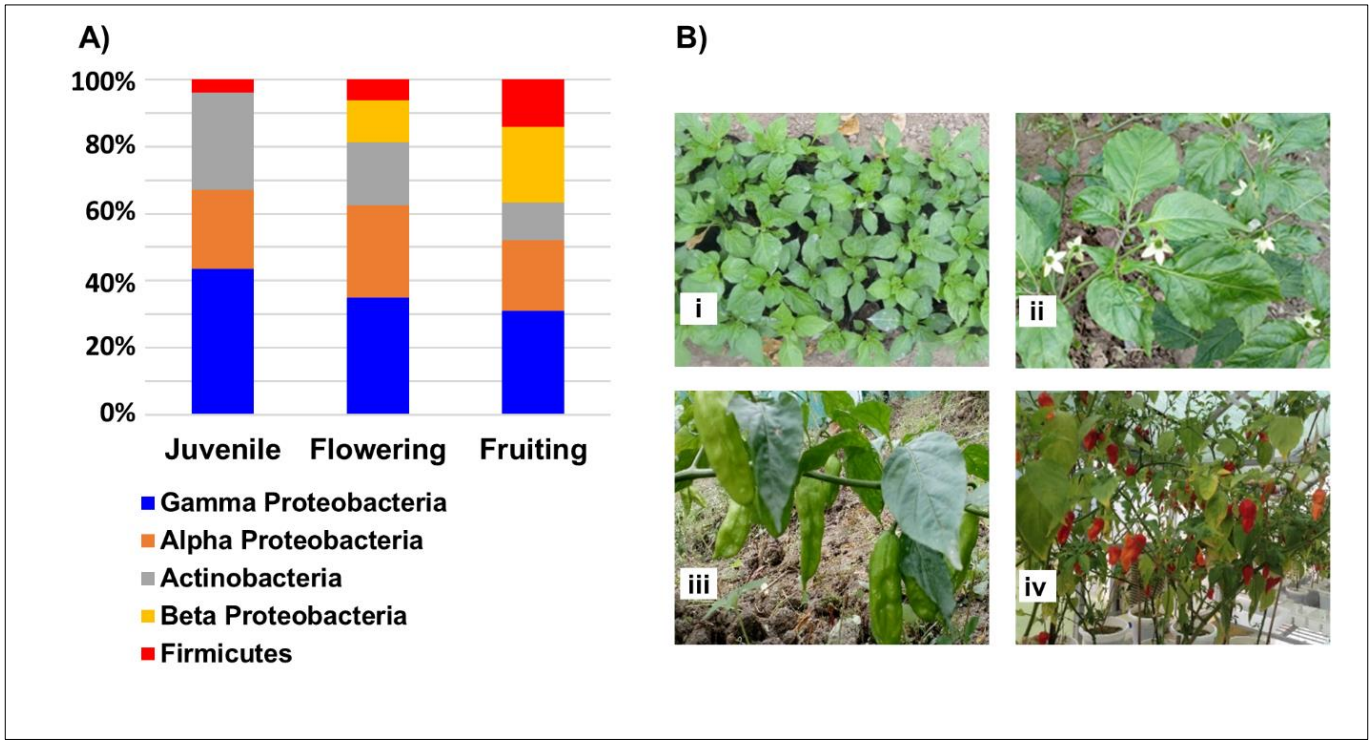

Fig 1: Three different growth stages of umorok rhizosphere are being associated with different types of bacterial species; however, all the identified rhizobacteria were representatives of six classical phyla. A) More bacterial diversity was observed in flowering and fruiting stages with additional beta proteobacteria that were not showing up in the juvenile stage. In the comparative analysis, gamma proteobacteria emerged as the most abundant bacterial variety and Firmicutes were turned out to be least showed organisms in all the three stages of the plant life cycle. B) The juvenile (i), flowering (ii), fruiting (iii), and matured (iv) stages of the umorok plant

Determination for the production of different cell wall degrading enzymes and PGPR activity

The isolated rhizobacteria demonstrated wide range of trait activities in different levels in the biochemical testing, however represented 32 potential microorganisms' data in all the analysis. Many of them have showed multiple PGPR and BCA at different levels (Figure 2, 3 and Table S1). Among the isolates PK7, PT5, PB6 and PF100 highest protease activity while PF47, PP6 and PB6 exhibited the highest chitinase activity. The phosphate solubilization activity was significantly produced by the isolates PF47, PF100, PT42 and PS9. Maximum beta-1,3-glucanase, HCN and siderophore activity was produced by PF 100, PF 82 and PF 88 (Figure 2, Table S1). Among the total isolates 25 % of the population shows strong nitrogen fixation ability whereas IAA production was exhibited by 9%, of the total population. *Lysobacter enzymogenes* PF100 and *Pseudomonas* sp. demonstrated strongest nitrogen fixation ability whereas *Pseudomonas putida* PF86, *Enterobacter aerogenes* PS22, and *Agrobacterium tumefaciens* PF34 showed satisfactory IAA production (Figure 3, Table S1).

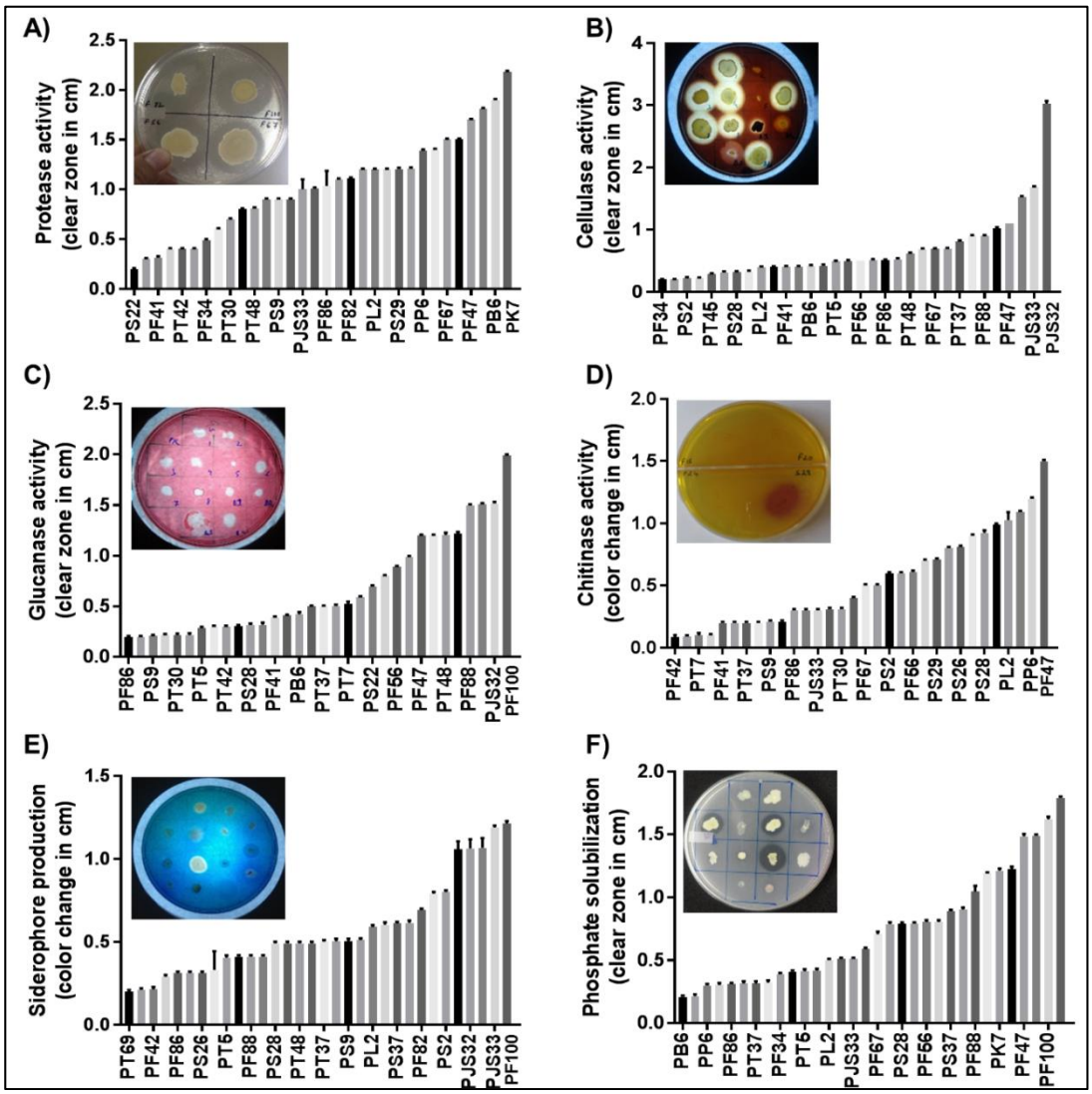

Fig 2: Biochemical test results of 32 potential umorok rhizobacteria along with characteristic biochemical activity clear zone, colony morphology, and color of media in agar plates: A) protease activity B) cellulase activity C) glucanase activity D) chitinase activity E) siderophore production and F) Phosphate solubilization

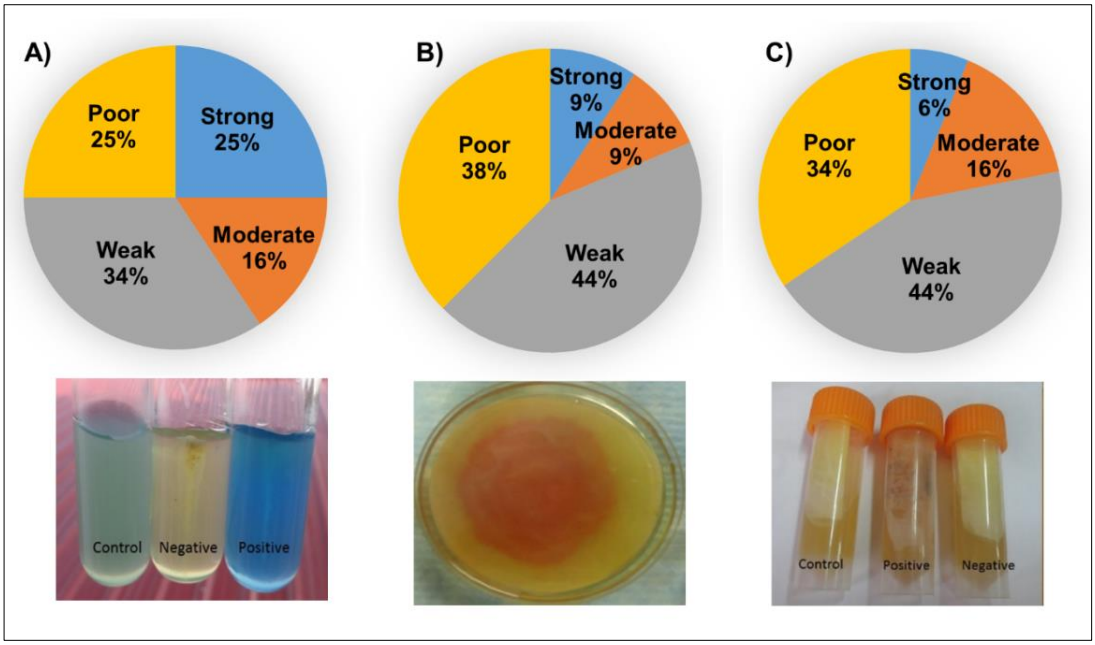

Fig 3: Qualitative biochemical tests performed on umorok rhizosphere to demonstrate the action potential based on characteristic color change: A) Nitrogen fixing assay revealed 25% of the assayed bacteria exhibited strong activity, and the color score was attributed based on the blue color intensity observed at the end of the assay, B) IAA production assay showed 9% of the bacteria exhibited strong IAA production, and the activity was measured based on the characteristic red color zone around the rhizobacterial colony, and C) HCN production assay witnessed 6% of the strong bacteria among tested microorganisms based on the color change from yellow to brown or reddish brown.

Antagonism Assay

The pathogen antagonist property of the umorok rhizobacteria was assessed using dual culture method against the most common soil and seed borne fungal pathogens *Pythium ultimum*, *Rhizoctonia solani*, and *Fusarium oxysporum* by co-culturing with the rhizobacteria. A significant inhibition of plant pathogens was observed when compared to the control plate (Figure 4). *Pythium ultimum* and *Fusarium oxysporum* were inhibited 38% (Fgure 4A) and 40% (Figure 4C) respectively when co-cultured with *Burkholderia cepacia* PK7, while *Rhizoctonia solani* was inhibited 36% with *Burkholderia metallica* PS2 (Figure 4B); for more information see the supplementary Table S2.

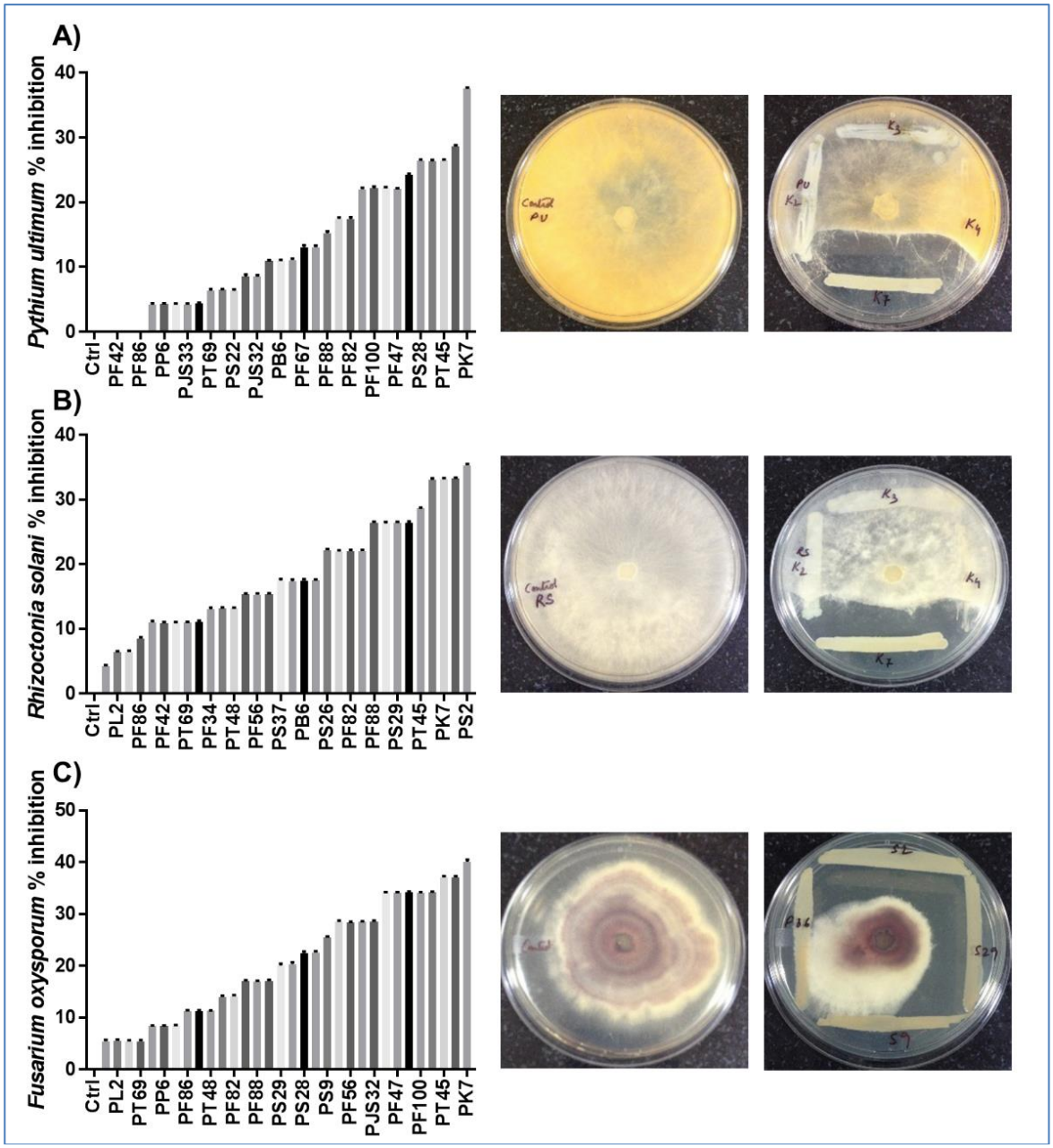

Fig 4: The dual culture of common fungal pathogens with umorok rhizobacteria exhibited significant pathogen inhibition, and zone of antagonistic inhibition is shown in the PDA plates: A) against *Pythium ultimum* B) against *Rhizoctonia solani* and C) against *Fusarium oxysporum*

Green House Experiment

The physical growth and the fruit yield of the umorok plant was measured after treating with selected inoculum of the 10 potential umorok indigenous rhizobacteria. The physical growth assessing parameters like root length, shoot length, total plant length from root to shoot, root dry weight, shoot dry weight, and number of fruits produced were quantitatively measured to see the effect of the rhizobacteria. Interestingly, all the groups were exhibited distinctly high yield when compared with the control. Statistical ANOVA analysis revealed the statistical significance of p value <0.0001 with all the groups when compared to the control group (Figure 5). Interestingly, all the physical parameters; root length (14.5 cm), shoot length (55.7 cm), plant length (70.3 cm), root dry weight (4.8 g), shoot dry weight (125.4 g), and number of fruits per plant (53); exhibited significant yield with *Burkholderia cepacia* PK7; however, *Burkholderia* ambifaria PR4 turned out to be as efficiant as *Burkholderia cepacia* PK7 in root dry weight (Figure 5A-F); for more details see the supplimentary Table S3.

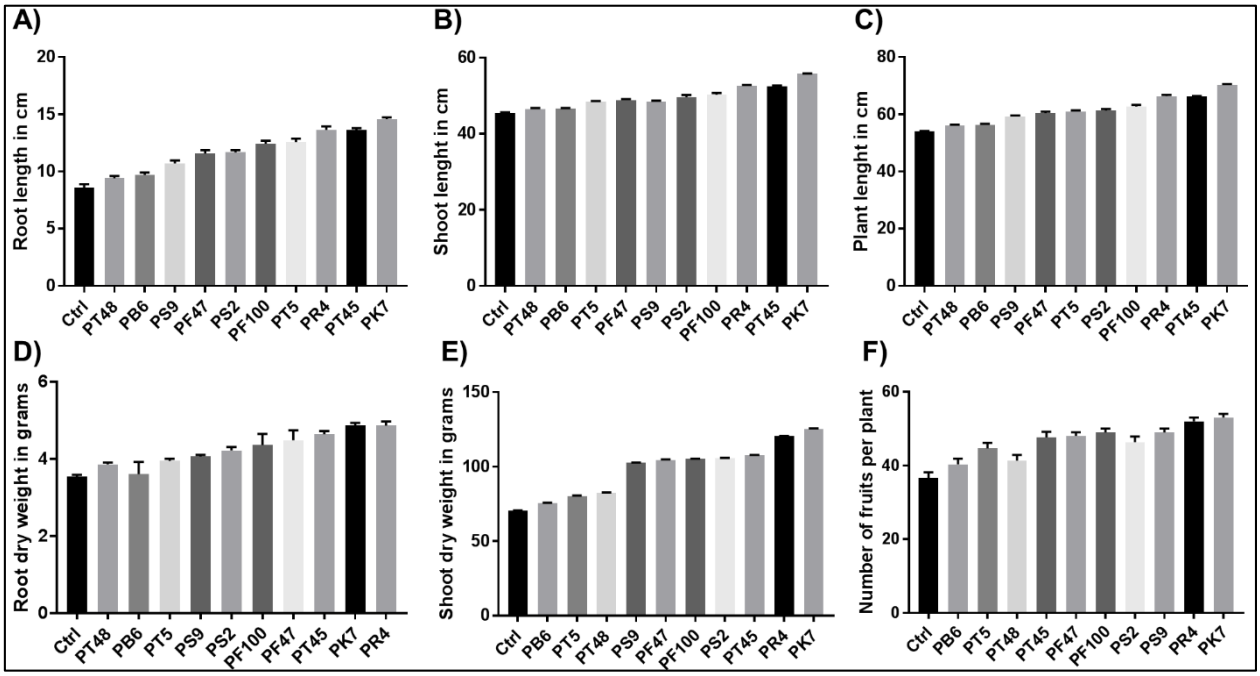

Fig 5: Plant growth and yield indicating physical parameters were quantitatively measured when umorok plant growing on sterile soil is inoculated with different PGPR that was isolated and characterized: A) root length, B) shoot length, C) plant total length from root to shoot, D) root dry weight, E) shoot dry weight, and F) number of fruits harvested were measured after the plant was grown with PGPR, and *Burkholderia cepacia* PK7 emerged as an efficient rhizobacterial strain though other strains also exhibited statistical significance ($p< 0.0001$) when triplicate data was subjected to ANOVA.

Discussion

Umorok (King chili) is a commercially important spice crop grown in the northeastern India which plays a critical role in its economy therefore, the study of plant growth promoting bacteria offers the insights into the crucial microorganisms that are essential for the plant growth and yield. Although previous studies delimited to series of biochemical analysis, identification of microbial strains, and cross species rhizobacteria validations, this study comprehensively unveiled the biochemical and molecular characteristics of the umorok PGPR. The plant growth promoting rhizobacteria (PGPR) of umorok were comprehensively characterized using series of biochemical and molecular identification tests. The potentially bioactive microbes were further inoculated to the rhizosphere of the chili plant to validate overall growth. Additionally, the outstanding PGPR that were reported in biochemical analysis were evaluated against common plant fungal pathogen using dual culture plate antagonism assays. While the total rhizosphere bacterial analysis revealed the presence of five phyla including alpha, beta, and gammaproteobacteria, actinomycetes, and firmicutes; gammaproteobacteria dominated the umorok rhizosphere in juvenile, flowering, and fruiting stages though little fluctuation in the percentage is observed (Figure 1). Because we isolated pure cultures of all most all the rhizosphere bacteria unlike other reported methods on chili varieties, where other researchers focused on either biochemical properties or limited to selective bacteria of PGPR (Shruthi 2017) [35] but not on systematic phyla analysis and their real contribution in the plant growth, thus the individual contribution of each bacterial species of umorok rhizosphere was assessed, and the findings are substantiated by recently reported rhizobacterial phyla in different parts of the geographical regions, soil types, and plants (Fierer, MA and RB 2007; Pascual *et al.* 2016; Colin *et al.* 2017; Wei *et al.* 2017) [13, 31, 39] though species level variation is inevitable.

Biochemical activities like protease, cellulase, glucanase, chitinase assays were accomplished across the rhizosphere; potential and statistically significant bacteria were shown in Figure 2A-D; interestingly, all are either beta (*Burkholderia cepacia* PK7) or gammaproteobacteria (*Pantoea eucrina* PJS32, *Lysobacter enzymogenes* PF100, and *Lysobacter enzymogenes* PF47). The results are not surprising as our results demonstrated the abundance of gram negative gammaproteobacteria, and these reported phyla are completely agreement with the previous studies of similar kind of soil type (Wei *et al.* 2017) [39] however bacterial species and strains identified here in this study are distinct to the umorok plant. In addition to biocontrol related biochemical activities, plant growth promoting related biochemical tests were also performed, and our quantitative results unveiled the *Lysobacter enzymogenes* PF100, a gammaproteobacteria, bacillus, and pseudomonas sps are the potential iron uptaking and phosphate solubilizing bacteria (Figure 2E,F). Despite the fact that *bacillus* and *pseudomonas sps* are siderophore producing and phosphate solubilizing bacteria (Adnan *et al.* 2017; Arora and Verma 2017) [1, 6], we reported a novel gammaproteobacteria in the umorok rhizosphere and that is as efficient as the reported bacteria (Table S1). Nitrogen fixing, IAA, and HCN producing bacterial efficiency was qualitatively analyzed: *bacillus*, *lysobacter*, and *pseudomonas* species contributed potentially in nitrogen fixing, *pseudomonas*, *enterobacter*, and *agrobacterium* found to exhibit efficient IAA production, while *enterobacter* and *lysobacter* turned out to be potential HCN producing rhizobacteria (Figure 3); the results are consistent with the reported results (Ahmad, Ahmad and Khan 2008) [3], nevertheless *lysobacteria* remain novel in our study. However, the study limits to the few microorganisms as the selective media is essential to isolate every unique species like *azotobacteria*, very frequent nitrogen fixer across the species and here it was not screened, thus results should be considered with little attention.

Biochemical testing result is the indispensable criteria for the selection of the lead PGPR for the assessment of antagonistic assays. When the antagonistic effect of PGPR was assessed, *burkholderia sps* and *lysobacter sps* were outperformed if the plant pathogens *Pythium ultimum, Rhizoctonia solani, and Fusarium oxysporum* were cocultured in dual culture plates (Figure 4); the results were similar to the reported results but *lysobacter* was unique in this set of results (Tariq *et al.* 2017) [37].

Considering the biochemical test results and antagonistic assay results, we enlisted promising PGPR of umorok that truly implicate in the overall growth of the plant. The central part of the study is the validation of the identified microorganisms, and they were inoculated in the plant soil to measure the growth indicating parameters including root length, shoot length, total plant length, root dry weight, shoot dry weight, and number of fruits for plant, most important yield parameter. If the results were when compared with the control all the top ten selective microorganisms showed statistically significant effect at $p< 0.0001$, but *Burkholderia cepacia* PK7 appeared to be outstanding, while *pseudomonas, bacillus* and *lysobacter sps* also exhibits strong plant growth promoting ability which have a future prospect for the development of microbial consortia for the overall umorok productivity in sustainable agriculture.

Umorok is indigenous to northeast region of India, geographical and climate conditions are unique to this region therefore these findings are likely to be suitable for the other plant species in the same region, nevertheless, the PGPR phyla reported typically consist of similar to other rhizobacteria in general but microbial strains are very specific. In this study, gammaproteobacteria seemingly dominated the juvenile, flowering and fruiting stages, and *lysobacter* was the novel organism that was observed in all the biochemical, antagonism, and plant growth parameter estimation studies. The study signifies that *Burkholderia sps* and *Lysobacter enzymogenes* are potential microbes out of all PGPR of umorok. Besides, *pseudomonas* and *bacillus* were also exhibited substantial contribution in the plant growth promotion and yield potential.

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